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Combined effects of sodium chlorite dip treatment and chitosan coatings on the quality of fresh-cut d'Anjou pears

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ABSTRACT

This study evaluated the effects of sodium chlorite (SC) alone and its sequential treatment with edible coatings on browning inhibition and quality maintenance of fresh-cut d'Anjou pears. Edible coatings were prepared from chitosan (CH) and its water-soluble derivative carboxymethyl chitosan (CMCH), separately. Pear wedges were immersed in SC solution, followed by coating with CH or CMCH solutions. The samples were packed in unsealed bags and stored at 4 °C for subsequent color, firmness, and weight loss measurement. The effects of the SC and coating treatments on polyphenol oxidase (PPO) inhibition and microbial inactivation were also evaluated. Results indicated that SC exhibited significant ($P < 0.05$) inhibition of browning and PPO activity. The SC treatment was also strongly effective in inactivating *Escherichia coli* O157:H7 on pear slices. Coating SC-treated pear slices with CH adversely affected the quality of pear slices by accelerating the discoloration of cut surfaces and increasing the PPO activity. On the contrary, coating SC-treated samples with CMCH significantly prevented the browning reaction and inhibited PPO activity. In addition, SC and CH/CMCH coatings maintained tissue firmness and did not affect weight loss. Our study may provide a scientific basis for the use of SC + CMCH treatment as an alternative preservation treatment for fresh-cut fruits and vegetables.

1. Introduction

Increased demand by modern consumers for natural, fresh, healthy and nutritious prepared foods has stimulated rapid expansion of the fresh-cut fruit and vegetable market over the past twenty years (Oms-Oliu et al., 2010; Shah and Nath, 2006). However, mechanical operations during minimal processing, such as peeling, cutting, slicing, and chopping, cause damage to the fruit tissues and initiate enzymatic reactions, resulting in cut-surface discoloration, tissue softening, water loss, aroma and flavor loss, off-flavor development, as well as microbial growth and spoilage (Oms-Oliu et al., 2010). These quality loss and safety concerns in fresh-cut produce drastically shorten shelf life, increase costs and affect consumer acceptance.

Edible coatings, a new strategy to extend shelf life and improve food quality of whole fruits and fresh-cut fruits, have been applied to many products. They can provide a selective barrier to moisture, oxygen, and carbon dioxide gas transfer, which slows ripening, reduces moisture loss, and helps to maintain fresh aroma and flavor (Olivas and Barbosa-Canovas, 2005). Edible coatings are also used as carriers of active ingredients, such as anti-browning, antimicrobial, and texture enhancing compounds, as well as flavors and nutrients, to improve the quality, safety, and nutritional value of fresh-cut fruits (Rojas-Grau et al., 2009).

Chitosan (CH) is a natural, non-toxic, biodegradable polymer that has been generally recognized as safe (GRAS). Chitosan has high molecular weight and low immunogenicity and is the N-deacetylated form of chitin mostly found in the exoskeletons of crustaceans, insects, and fungi (Luo et al., 2010). Its film-forming and antimicrobial properties lend CH promise as an edible coating to delay senescence and maintain the quality of fresh-cut fruits and vegetables (Tamer and LCopur, 2009). However, due to its insolubility at neutral pH, the application of CH is restricted to some extent (Ge and Luo, 2005). An amphiprotic water-soluble and nontoxic (Kennedy et al., 1996) derivative of CH formed by carboxylation, N,O-carboxymethyl chitosan (CMCH), has been shown to have beneficial effects in delaying ripening of whole fruits when used soon after harvest (Meheriuk and Lau, 1998). Although CMCH has not been approved as a GRAS substance by FDA, it has been investigated intensively and found nontoxic *in vitro* and *in vivo* (Kennedy et al., 1996). Previous studies have reported that coating apples with CMCH films aided in maintaining their quality during cold

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storage for more than 6 months (Davis et al., 1998). Huanghua pears coated with CMCH significantly decreased respiration rate, reduced weight loss, and delayed senescence, compared with the uncoated pears (Zhou et al., 2008). However, no literature could be found on applying CMCH on fresh-cut fruits. This study investigated the abilities of CH and CMCH to maintain quality of fresh-cut pears.

Sodium chlorite (SC) is a well-known antimicrobial agent, due to its strength as an oxidizing agent. Under acidic conditions, it can generate chlorine dioxide gas, which is also a powerful oxidizing agent and has been used to sterilize equipment and food preparation surfaces, prevent and remove biofilms, bleach paper, textiles, flour and disinfect water (Lu et al., 2006). Recent studies have shown that SC also effectively inhibits enzymatic browning of fresh-cut apples (Lu et al., 2007). Therefore, SC has a dual effect on browning inhibition and pathogen inactivation (He et al., 2008).

Pear is a popular component of salad bars, snack bars, or other catering services in the USA. Fresh-cut pears have very high susceptibility to enzymatic browning and tissue softening after cutting (Sapers and Miller, 1998). Recently, several studies have used chemical dip treatments with or without edible coating in the preservation of fresh and fresh-cut pears, in order to prevent cut-surface discoloration, maintain tissue texture, reduce moisture loss and inhibit the growth of microorganisms (Bai et al., 2009; Lin and Zhao, 2007; Xiao et al., 2010).

The strong efficacy of sodium chlorite both as a sanitizer and as a browning inhibitor on fresh-cut apples suggests that it might also be effective on fresh-cut pears. Additionally, CH and CMCH are proposed edible coating materials due to their film-forming and antimicrobial properties. Therefore, the objective of our research was to investigate the effectiveness of sodium chlorite with and without chitosan or N, O-carboxymethyl chitosan in maintaining the quality of fresh-cut d'Anjou pears during storage.

2. Materials and methods

2.1. Materials

Green d'Anjou pears (*Pyrus communis* L.) used in this study were purchased at a local grocery in Greenbelt, MD, USA, and stored at 4 °C before processing. The fruit were sorted to ensure that samples with uniform size, color, and shape used for the experiments. Sodium chlorite (SC) and other chemicals (acetone, acetic acid, Triton X-100, sodium acetate, phosphate buffered saline, etc.) were purchased from VWR (West Chester, PA, USA), chlorogenic acid from Sigma–Aldrich (St. Louis, MI, USA) and trypticase soy broth from Difco (Corpus Christi, TX, USA). Low molecular weight chitosan (Sigma–Aldrich, St. Louis, MI, USA), with a deacetylation degree of 92%, and N,O-carboxymethyl chitosan (Nantongxingcheng, Nantong, Jiangsu Province, China), with a deacetylation degree of 96%, carboxylation degree of 65% and molecular weight of 67–79 kDa, were used to prepare edible coating solutions. All chemicals used in this study were analytical grade and solutions were prepared using distilled water.

2.2. Preparation of edible coating solutions

Chitosan (CH) coating solutions (1%, w/v) were prepared by dissolving chitosan in an aqueous solution of 1% (w/v) glacial acetic acid, and stirred overnight until chitosan was completely dissolved. N, O-carboxymethyl chitosan (CMCH) aqueous solutions were prepared by dispersing N, O-carboxymethyl chitosan into distilled water at a concentration of 1% (w/v) and stirring for 1–2 h. The final pH values of CH and CMCH were 4.0 and 6.4, respectively.

2.3. Sample preparation

Pears were rinsed under running tap water, dried with paper towels, and then hand-peeled. They were cut into different shapes for different measurement purposes using sterilized sharp stainless steel knives: cut vertically into equal wedges (ca. 5 mm thick) for color measurement and cut transversely into equal cubes (ca. 20 mm thick) for firmness and weight loss evaluation. The pear wedges/cubes were immediately dipped in the SC treatment solutions (i.e. 0, 300, 600, or 1000 mg/L) for 2 min. Samples were then drained for 1 min, dried in the air for 30 min, and packed into unsealed plastic bags. Samples receiving edible coating treatments additionally were immersed in the CH or CMCH solutions for 2 min, drained for 1 min and dried in the air for 30 min before the packing step. After drying, each of the samples treated with CH gained weight of 0.11 ± 0.01 g; for CMCH coated samples, each piece gained weight of 0.29 ± 0.01 g. The analyses were performed on 0, 1, 3, 7, and 10 d of storage at 4 °C.

2.4. Color measurement

Color (CIE L^*, a^*, b^*) was directly measured in a total of 3 locations on both cut surfaces of each slice with a ColorFlex colorimeter (Hunterlab, Reston, VA, USA), and the mean value was taken to ensure the color readings were representative of each slice. The equipment was set for D65 illuminant and 10° observer angle and calibrated with standard white and black plates. Together, five pear slices in each of two replicate packages were evaluated for each treatment on each sampling day. The results were expressed as L^* and a^* values.

2.5. Fourier Transform Infrared Spectroscopy (FT-IR) analysis

To investigate the reaction between CH/CMCH and SC further, FT-IR analysis was carried out. The FT-IR spectra of CH, CH + SC, CMCH, and CMCH + SC were recorded using an FT-IR spectroscope (Jasco 4100, Jasco Inc., Easton, MO, USA) with an attenuated total reflection (ATR) cell. Samples were first cast-dried on an aluminum tray overnight, and then mounted onto ATR crystal directly. The spectra were acquired in the whole mid-infrared region (4000–800 cm^{-1}) at a 4 cm^{-1} resolution.

2.6. Firmness evaluation

Firmness was assessed using a texture analyzer (TA-XT2, Texture Technologies Corp., Hamilton, MA, USA) to measure the maximum force required to penetrate pear cubes (ca. 20 mm thick) to a depth of 5 mm with a 5 mm diameter rod at the speed of 2 mm/s (Guan and Fan, 2010). The peak force was recorded and used to represent firmness for each sample. During each evaluation, three pear cubes from each of the two packages were tested for each treatment and the average peak force per treatment was computed.

2.7. PPO inhibition

Pear polyphenol oxidase (PPO) was extracted from pear acetone powder according to a previously reported method (Yemenicioglu et al., 1997) with minor modifications, which is described below in detail. Acetone powder was prepared from peeled pear pulp. Small pieces of peeled pear pulp (200 g) were homogenized in a pre-chilled blender with twice the weight of cold acetone (−20 °C, 400 mL), and then filtered through a Buchner funnel using Whatman No. 1 filter paper under vacuum. The residue was re-extracted

at least 3 times with 200 mL of cold acetone. The resulting white powder was vacuum-dried at room temperature (22 °C) and stored in vacuum bags at −20 °C until used for PPO extraction.

For PPO extraction, 1 g of pear acetone powder was incubated with 50 mL of 0.1 M KH_2PO_4/Na_2PO_4, pH 7.2 buffer containing 1% Triton X-100 for 20 min while stirring with a magnetic stirrer at 4 °C, followed by centrifugation for 30 min at 6000 × *g*. The supernatant was filtered using Whatman No. 1 filter paper. The PPO extract was stored at −20 °C and was discarded if visible precipitation (due to the enzyme denaturation) or dark color (due to the phenolic oxidation) was observed after it was thawed.

PPO inhibition was evaluated by measuring pear PPO activity with and without inhibition solutions (300, 600, or 1000 mg/L SC solution and/or 1% CH or CMCH solution). Enzyme activity was determined by the spectrophotometric method at 420 nm using chlorogenic acid as the substrate. The reaction solution for PPO inhibition consisted of 2.35 mL of 0.1 M sodium acetate–acetic acid buffer (pH 4.2), 0.3 mL of 0.05 M chlorogenic acid, 0.3 mL of inhibition solutions (300, 600, or 1000 mg/L SC solution and/or 1% CH or CMCH solution) and 0.05 mL enzyme extract, while the control was prepared by replacing the inhibition solution with acetate–acetic acid buffer.

Enzyme activity was based on the initial reaction rate. One unit of enzyme activity was defined as t increase in absorbance of 0.001 per minute at 25 °C. The degree of inhibition was expressed as percent inhibition using the formula $[100(A - B)/A]$, where A and B stand for enzyme activities in the control and inhibitor treatments, respectively.

2.8. Microbial inactivation

A strain of green fluorescent protein (GFP)-expressing *Escherichia coli* O157:H7 was obtained from Environmental Microbial and Food Safety Laboratory of United State Department of Agriculture (Beltsville, MD, USA). *E. coli* culture was grown overnight in 30 mL trypticase soy broth (TSB) supplemented with ampicillin at 37 °C. Inoculum for experiments was prepared by centrifugation of the culture at 6000 × *g* for 5 min at 4 °C. Supernatant was discarded and bacteria were resuspended in sterile phosphate buffered saline (PBS) solution. The process was repeated twice. The inoculum was obtained by diluting the *E. coli* cells in sterile distilled water to obtain a concentration of around 10^5 CFU/g.

Microbiological analysis was conducted on 3 replicate samples consisting of 4 uniform 5 g pieces of d'Anjou pear cut from pear wedges for each treatment. Samples (20 g) were immersed in the *E. coli* inoculum solution for 2 min. After inoculation, fruit were drained for 1 min and dried in the air for 30 min at room temperature. Then, the inoculated pears were dipped into water or SC solutions (300, 600, or 1000 mg/L) for 2 min, followed by draining for 1 min and drying for 30 min. Samples receiving edible coating treatments were additionally immersed in CH or CMCH solutions at 1%, w/v for 2 min before draining for 1 min and drying for 30 min.

The treated samples were placed in sterile stomacher bags containing TSB and homogenized for 1 min in a Seward stomacher 400 (Bohemia, NY, USA). *E. coli* populations were enumerated using a most probable number (MPN) method (Luo et al., 2011). MPN blocks were prepared by adding 2.7 mL TSB medium into each well. Sample diluent (0.3 mL) was added to the first cell of each column, followed by serial dilution. The blocks were incubated at 37 °C for 24 h and the numbers of positive cells for each column were recorded. The *E. coli* concentration was calculated from this data using a software MPN calculator (Curiale, 2004) available online. The results were expressed as the $\log_{10}$ reduction of the population after treatment and the assay was conducted in triplicate.

2.9. Weight loss

Weight loss was measured at different storage times by weighing the bags containing six pear cubes and was calculated as follows:

$$\frac{(\text{weight at day } 0 - \text{weight at day } N) \times 100}{\text{weight at day } 0}$$

where N represents the number of days of storage when the bag was weighed. Three replicate samples were measured for each treatment on each sampling day and the results were expressed as the percentage loss of initial weight. The samples for weight loss analysis were monitored for 10 d.

2.10. Statistical analysis

The experiment was conducted using a factorial design. Data were analyzed as a two-factor linear model using statistical analysis software (SPSS 13.0) with storage time and treatment as the factors. Microbial data were log transformed. One-way analysis of variance (ANOVA) was conducted to check the normality and variance homogeneity of the linear models. The statistical significance of the data was determined by performing Tukey's honestly significant difference (HSD) tests for post hoc multiple comparisons at an experiment-wise significance level of 0.05. Two replicates of 5 slices each were used for color measurement and two replicates of 3 cubes each were used for texture measurement. Three replicates were used for PPO inhibition, weight loss and microbial inactivation.

3. Results and discussion

3.1. Color

3.1.1. Changes in L^* value

In the CIE $L^*a^*b^*$ color space, L^* value represents lightness. The higher the L^* value, the brighter the surface. In general, L^* tended to decrease with increasing storage. The effect of SC and coating alone, or their combination treatments on L^* value of fresh-cut pear slices during storage was shown in Fig. 1.

From Fig. 1A, it can be seen that without SC treatment, the L^* value decreased significantly ($P < 0.05$) from 75 to 71 during the 10-day storage. Treatment with 300 mg/L SC had little effect on L^* value, suggesting that enzymatic browning was not inhibited. Moreover, from day 3 until the end of storage, 300 mg/L SC treatment accelerated the decrease in L^* value of pear slices compared to the control. However, the L^* values of samples treated with 1000 mg/L SC remained significantly higher than the control during the entire 10 d of storage and those for 600 mg/L remained higher until day 7, after which they decreased to below the level of the control. These results indicate that enzymatic browning of fresh-cut pears can be successfully inhibited by higher concentrations of SC dip treatment. Similar results have also been reported by other researcher (Lu et al., 2007), who found that SC could inhibit browning of fresh-cut apples. The dramatic decrease in L^* value of pear slices treated with 300 mg/L SC after day 2 and 600 mg/L SC after day 7 are consistent with a previous report, which pointed out that SC delayed browning initially, but that the inhibition was short-lived (Guan and Fan, 2010). One proposed reason for this temporary effect is that the initial effect of SC to lighten the color is a bleaching reaction. The subsequent browning effect may be due to the oxidation of endogenous browning inhibitors, enzymatic browning substrates or intermediate products.

Fig. 1B showed that the L^* value of all the samples coated with CH (with or without prior dipping in SC solution) decreased dramatically during the storage. This result indicates that the use of

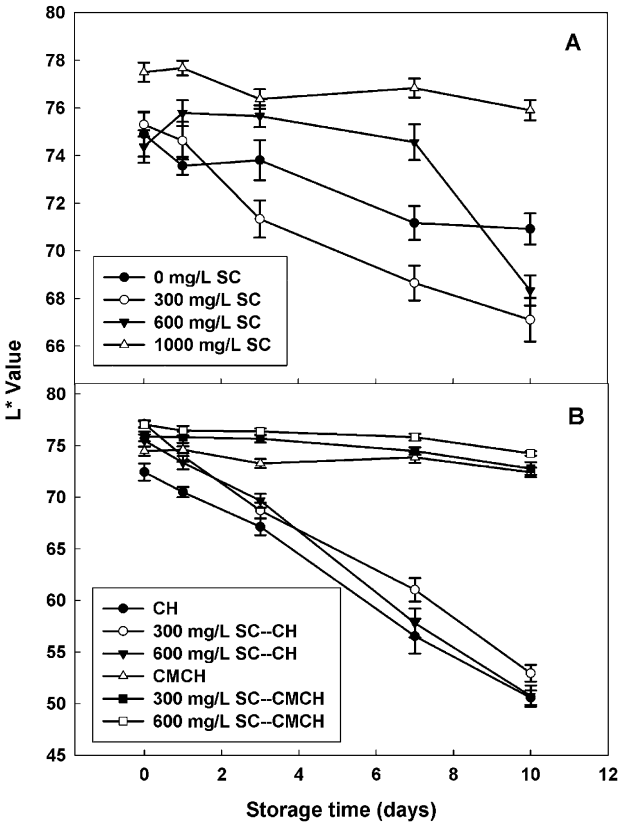

Fig. 1. Effect of sodium chlorite (SC; panel A) and edible coatings (CH/CMCH; panel B) on the changes of L^* value of fresh-cut pear slices stored at 4 °C. Vertical bars represent standard errors ($n = 10$).

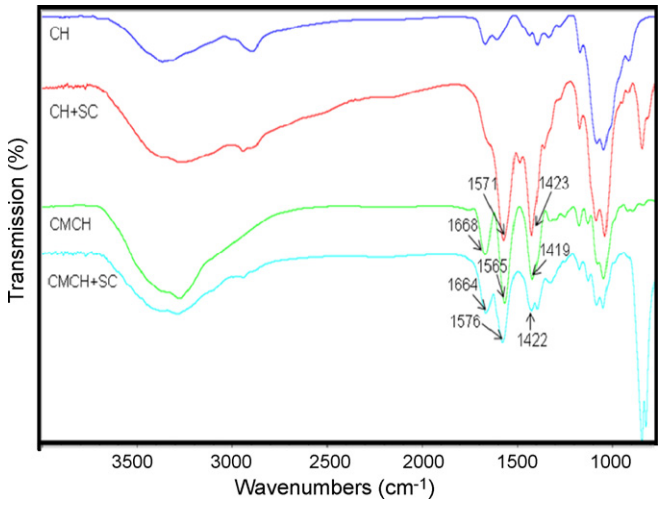

Fig. 2. FT-IR spectra of chitosan (CH), chitosan + sodium chlorite (CH + SC), N,O-carboxymethyl chitosan (CMCH) and N,O-carboxymethyl chitosan + SC (CMCH + SC).

CH coating does not prevent enzymatic browning of the fresh-cut pears; conversely, it accelerates their surface discoloration. This result is in accordance with a recent study showing that chitosan decreased the L^* value of fresh-cut mango in storage (Plotto et al., 2010), but is contrary to many other reports, which demonstrated that CH was effective for inhibiting browning. For example, Worakeeratikul and others reported that CH retarded browning and maintained the L^* value of fresh-cut rose apple (Worakeeratikul et al., 2007); Pen and others reported that application of chitosan coating delayed discoloration of fresh-cut Chinese water chestnut (Pen and Jiang, 2003); and, Xiao and others showed that the use of CH coating on fresh-cut pears significantly benefitted color retention (Xiao et al., 2010). The combination of SC and CH had no apparent benefit in maintaining the quality of the fresh-cut pears when compared with SC treatment alone and actually had some deleterious effects, including tissue injury and exacerbated browning. It has been reported that acetic acid was effective at reducing lettuce-butt discoloration during storage and commercial handling (Castañer et al., 1997), suggesting that the browning acceleration was not due to the presence of acetic acid in CH coating solution. This phenomenon may be related to the oxidation of CH by SC under acidic conditions, a recent discovery reported by Murinov et al. (2010). This reaction involves the oxidation of chitosan's $-CH_2OH$ into $-COOH$, leading to the formation of an amide bond between the amino nitrogen atom and carbon atom of the carboxyl group. Acylamino groups can be formed both intermolecularly and intramolecularly. Consequently, any capability of SC treatment to inhibit browning is nullified by its reaction with CH. The acidity of the solution may also have been responsible for the tissue damage and aggravated browning observed in these samples. However, the CMCH solution had pH of 6.4 and thus CMCH did not react with SC to compromise its anti-browning effect. Furthermore, since CMCH is the carboxymethylation product of CH and some of its amino groups are already attached to carboxyl groups, intermolecular and intramolecular acylamino groups cannot be as readily formed in CMCH as they can in CH.

From Fig. 1B, it also can be observed that samples treated with CMCH coating alone or in combination with different concentrations of SC retained significantly higher L^* values than any other treatments except for 1000 mg/L SC during the 10-d storage. This result suggests that the use of a CMCH coating significantly inhibits browning on fresh-cut pears. CMCH is a carboxymethylated derivative of CH, in which the carboxymethylation reaction happened in the hydroxymethyl and amino groups of the CH chain. Although there are no related reports on the use of CMCH on fresh-cut produce, CMCH has been shown to be effective in maintaining freshness of whole Huanghua pears and flower betony (Yi et al., 2005; Zhou et al., 2008). These reports support our findings that CMCH helps to maintain the color of fresh-cut pears. Fig. 1B also documents that CMCH, when used along with lower concentrations of SC (300 and 600 mg/L), prevented pear slices from obvious browning during 10 d of storage, which was a similar result to that observed using the higher concentration of SC (1000 mg/L) alone. Therefore, the combination of SC and CMCH may be a better choice for application on fresh-cut produce.

To attempt to understand why SC + CH did not inhibit enzymatic browning while SC + CMCH did demonstrate a significant anti-browning effect on fresh-cut pear slices, FT-IR analysis of SC + CH and SC + CMCH was carried out and the results are presented in Fig. 2. From the spectrum of SC + CH, several changes were observed with respect to the spectrum of CH. New absorption bands appeared at 1423 cm^{-1} and 1571 cm^{-1}, which is consistent with Murinov's report on CH oxidation with SC (Murinov et al., 2010). The appearance of the strong absorption band at 1423 cm^{-1} can be attributed to carboxyl groups and was assigned to the interaction of C–O stretching vibrations and in-plane bending vibrations of O–H groups (Xie et al., 2005). The absorption band at 1571 cm^{-1}, can be assigned to the amide II group of chitosan (Le-Tien et al., 2004), and is the result of covalent bonding between the carboxyl group and the amino group, indicating that the amide group formed intermolecularly and intramolecularly. Therefore, it can be concluded that the ineffectiveness of SC + CH on anti-browning is due to the oxidation reaction between SC and CH, which indirectly cancelled the anti-browning effect of SC. In the spectrum of CMCH, the absorption band at 1668 cm^{-1} corresponded to the amide I stretching of C=O, and the absorption band at 1565 was assigned to amide

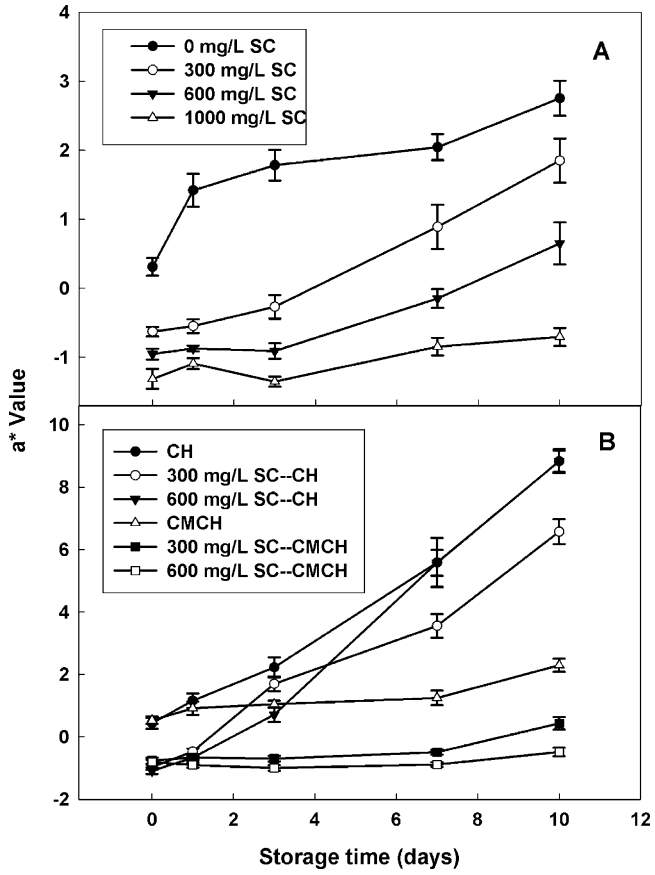

Fig. 3. Effect of sodium chlorite (SC; panel A) and edible coatings (CH/CMCH; panel B) on the changes of a^* value of fresh-cut pear slices stored at 4 °C. Vertical bars represent standard errors ($n = 10$).

II group. Meanwhile, the spectrum of CMCH showed a characteristic peak (1419 cm^{-1}) for symmetric stretching vibrations of carboxyl groups, suggesting that there were carboxymethyl groups existing on CMCH (Zhao et al., 2002). Compared to CMCH, a similar spectrum was observed for CMCH + SC, in which there were three corresponding bands at 1664 cm^{-1}, 1576 cm^{-1} and 1422 cm^{-1}, respectively. It suggested that there was no reaction between SC and CMCH. Consequently, CMCH + SC treatment possessed a very good anti-browning effect on fresh-cut pears.

3.1.2. Changes in a^* value

In the CIE $L^*a^*b^*$ dimensional coordinate system, the a^* value indicates the color change from green (negative values) to red (positive values). The high a^* values have been used to indicate browning of samples.

In our study, the a^* values of pear samples of all treatments tended to increase with increasing storage time, showing that these fresh-cut pear slices underwent browning. Among the control and SC treated samples, the a^* values of the pear slices treated with SC were significantly lower than that of untreated samples throughout the 10-d storage period (Fig. 3A). Also, the increase in a^* values of the pear slices was SC concentration-dependent; the higher the SC concentration, the lower the a^* value. Accordingly, treatment with 1000 mg/L SC yielded the lowest a^* value of all the treatment levels tested, consistent with the changes in L^* values. These results suggest that SC has the potential to inhibit the discoloration of fresh-cut pears and that the effectiveness of browning inhibition is concentration-dependant.

The a^* values of pear slices treated with the CH and CMCH coatings alone or combined with SC are shown in Fig. 3B. In general, samples subjected to treatments containing CH had significantly ($P < 0.05$) higher a^* values than their corresponding groups containing CMCH during storage, especially towards the end of storage. It was noted that pear slices treated with CH and CMCH alone both received significantly higher a^* values initially after cutting, compared to all other treatments with SC ($P < 0.05$). However, while the a^* values for CH treated samples continued to increase over time, the a^* values for CMCH treated samples remained relatively stable through the end of storage. It can be concluded that CH alone could not inhibit browning of fresh-cut pears, whereas, CMCH alone was able to reduce browning during storage. Among all treatments containing CMCH, the addition of either 300 mg/L or 600 mg/L SC substantially further improved browning inhibition as evidenced by the slower changes in a^* values during storage. These results for a^* values were consistent with the corresponding L^* values (Fig. 3A).

3.2. Firmness

In our study, the aim of texture evaluation was to determine the effects of SC, CH, and CMCH on firmness retention of fresh-cut pears during storage. Table 1 presents the firmness values of fresh-cut pear cubes during the 10-d storage at 4 °C. Due to variations in pear fruit maturity stage and storage time among different batches, the firmness of pear slices varied. Therefore, treatment comparisons on firmness are made based on the overall changes in firmness during the 10-d storage among treatments. In general, there was no significant ($P > 0.05$) loss in firmness during storage among all treatments. Interestingly, samples treated with 600 mg/L SC plus CMCH had a significant increase in firmness from day 0 (25.6 N) to day 10 (31.3 N).

Towards the end of the storage period, observations were made which indicate that firmness readings may have been affected by other factors besides the treatments: (i) noticeable sound could be heard when the texture analyzer probe punctured the pear surface; and, (ii) sponge-like structures with rough surface were observed. These phenomena were observed due to the dehydration of the pear tissue on the surface during storage, which led to a hardening of the pear wedge that increased the measured resistance, and consequently resulted in high firmness measurements. Therefore, these firmness data should not be used as a sole evidence to draw the conclusion that SC, CH and CMCH maintained the texture of fresh-cut pears and more experiments need to be carried out for this purpose. The dehydration of pear cubes was not surprising since they were stored in unsealed bags.

3.3. PPO activity inhibition

Inhibition of PPO activity by different treatments is shown in Fig. 4. All treatments containing SC significantly inhibited PPO activities, with stronger, although not statistically significant, inhibition observed with higher SC concentration. When used alone, or in combination with SC, CH treatment adversely affected PPO inhibition. Interestingly, when applied alone, CMCH significantly inhibited PPO activities. However, when combined with SC, the combined treatment slightly reduced PPO inhibition compared to the solitary SC treatment of the corresponding SC concentration. All of these results were consistent with the changes in color expressed as L^* and a^* values.

PPO is a copper-containing enzyme that catalyzes both the o-hydroxylation of monophenols and the oxidation of o-diphenols. It has been proved that copper ion is in the bivalent state and

Table 1
Firmness of fresh-cut d'Anjou pear cubes treated by sodium chlorite (SC) dip treatment with or without edible coatings (CH/CMCH) during the 10-d storage at 4 °C ($n = 6$). Values in the same day followed by the same lower case letter and in the same treatment followed by the same upper case letter are not significantly different ($P > 0.05$).

Treatment		Firmness (N)				
SC (mg/L)	Coating	0 D	1 D	3 D	7 D	10 D
0	None	20.9 ± 1.1aA	22.3 ± 0.8abA	20.2 ± 1.0aA	21.0 ± 0.3aA	21.1 ± 0.4bA
300		22.8 ± 1.1abA	21.3 ± 1.1aA	24.9 ± 1.3aA	23.4 ± 1.2aA	23.4 ± 0.6bcA
600		22.7 ± 0.6abA	23.0 ± 1.4abA	21.3 ± 1.0aA	21.2 ± 1.4aA	22.1 ± 0.4bA
1000		23.1 ± 1.2abcA	22.3 ± 0.6abA	20.3 ± 2.3aA	20.5 ± 0.3aA	17.7 ± 0.8aA
0	CH	27.3 ± 0.4dA	25.0 ± 1.5abA	25.9 ± 2.3aA	24.3 ± 2.6aA	24.7 ± 1.4bcA
300		26.3 ± 0.7bcdA	23.8 ± 2.3abA	23.5 ± 1.8aA	25.5 ± 1.7aA	27.0 ± 1.4bcA
600		25.0 ± 1.1bcdA	25.1 ± 2.0abA	25.1 ± 1.6aA	24.7 ± 1.8aA	26.6 ± 0.7cA
0	CMCH	27.2 ± 0.1cdA	27.6 ± 1.2bA	26.7 ± 1.3aA	27.5 ± 0.6aA	27.2 ± 1.1cdA
300		27.6 ± 0.5dA	25.5 ± 0.9abA	27.0 ± 1.3aA	26.2 ± 0.9aA	27.2 ± 1.3cdA
600		25.6 ± 0.6bcdA	24.6 ± 0.9abA	27.3 ± 1.7aAB	27.3 ± 1.1aAB	31.3 ± 0.5dB

that the catalytic activity of the enzyme is based on the change in valency, $Cu^{2+} \leftrightharpoons Cu^{+}$, as follows (Hoffmann-Ostenhof, 1954; Singer and Kearney, 1954):

$$2Cu^{2+}\text{-enzyme} + \text{o-diphenol} = 2Cu^{+}\text{-enzyme} + \text{o-quinone} + 2H^{+} \quad (1)$$

$$2Cu^{+}\text{-enzyme} + 1/2O_2 + 2H^{+} = 2Cu^{2+}\text{-enzyme} + H_2O \quad (2)$$

SC is an efficient oxidizing agent, which can oxidize Cu^{+} into Cu^{2+}. A previous report indicated that inactivation of the enzyme was associated with increase in Cu^{2+} concentration (Kertész et al., 1972). A recent study also proposed that SC may affect the oxidation state of copper so as to alter the catalyzing activity of PPO (He et al., 2008). Moreover, phenolic compounds existing in fruits, like chlorogenic acid and catechol, might be degraded by the oxidative effect of SC in acidic conditions, which might be another explanation for the inhibition of PPO.

Furthermore, it should be noted that the PPO inhibition percentages of SC + CMCH treatments were obviously lower than those of the SC treatments alone, which seemed to be inconsistent with the results of color parameters of L^* and a^* (discussed above). This discrepancy might be attributed to the decreased solubility of SC + CMCH in the testing reagent. It was observed that during the measurements, cotton-like precipitation was generated when CMCH solution was added to the cocktail of enzyme extract in the sodium acetate–acetic acid buffer (pH = 4.2). The CMCH molecule is known to contain both amino groups and carboxymethyl groups.

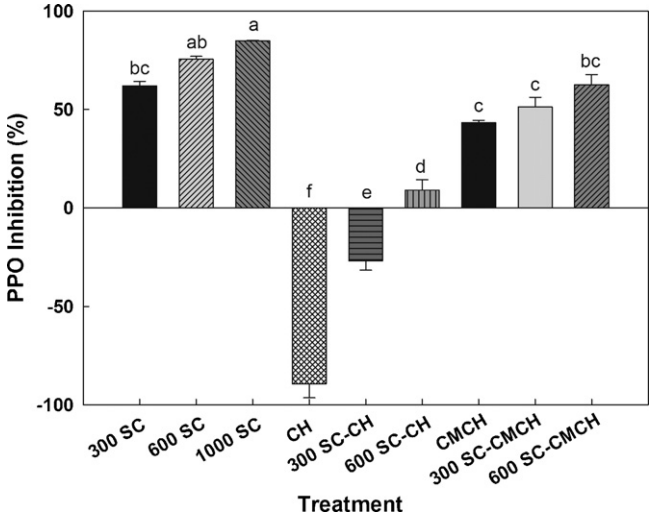

Fig. 4. PPO inhibition (%) of sodium chlorite (SC) with or without edible coatings (CH/CMCH). Values followed by the same lower case letter are not significantly different ($P > 0.05$). Vertical bars represent standard errors ($n = 3$).

These functional groups confer to CMCH the property of amphiprotic ionization. When the solution pH is equal to the isoelectric point (pI) of CMCH, precipitation of the molecules occurs. A recent reference reported that the ionization degree of carboxyl groups in the CMCH molecule decreased in acidic conditions and a large amount of hydrogel formed due to strong hydrogen bonds (Dong et al., 2008) between CMCH molecules. This reaction may explain the phenomenon we observed. Possibly, the formation of CMCH hydrogel may have interfered with the accurate measurement of PPO inhibition activity.

3.4. Weight loss

The weight loss of the uncoated and coated pears throughout the 10-d storage period is shown in Table 2. For all treatments, the weight loss steadily increased with storage time, indicated by the different upper case letters. On the 10th day of storage, samples treated with CH without SC had the lowest weight loss and no significant difference was shown compared with the control. Meanwhile, the weight loss of all other samples was higher than that of the control (i.e. pears with no treatment), suggesting none of the treatments were effective in reducing weight loss of fresh-cut pears, and most even accelerated the weight loss. As mentioned in the firmness results, sponge-like structures were observed, which were probably due to dehydration. Notably, the tendency for weight loss decreased slightly as the concentration of SC increased in the treatments.

These results were contrary to those reported in many other studies, which demonstrated that CH coating could reduce the weight loss of food products, such as pear wedges (Xiao et al., 2010; Zhou et al., 2008), fresh-cut papaya 'Maradol' (Gonzalez-Aguilar et al., 2009), and sliced mango fruit (Chien et al., 2007). However, one study reported that chitosan coating improved the efficiency of the osmotic dehydration process, increasing the water loss of papaya (García et al., 2010). In our study, CH coating application showed poor moisture barrier properties and did not reduce weight loss or prevent water evaporation from the cut surfaces in fresh-cut pears, probably due to CH's balanced hydrophobic and hydrophilic groups on its backbone (Neto et al., 2005). CMCH, which has strong hydrophilicity (Pang et al., 2007), adversely accelerated the water loss. One probable reason is that the samples after dipping and coating treatments were not dried completely in air before packing and the high initial humidity of the product could increase the apparent weight loss.

3.5. Microbial inactivation

The effect of the different treatments on microbial inactivation on fresh-cut d'Anjou pears is presented in Fig. 5. The initial *E. coli* population inoculated on pear slices was 4.6 ± 0.1 log CFU/g.

Table 2
Weight loss (%) of fresh-cut pears slices treated by sodium chlorite (SC) dip treatment with or without edible coatings (CH/CMCH) during the 10-d storage at 4 °C ($n = 3$). Values in the same day followed by the same lower case letter and in the same treatment followed by the same upper case letter are not significantly different ($P > 0.05$).

Treatment		Weight loss (%)			
SC (mg/L)	Coating	1 d	3 d	7 d	10 d
0	None	1.89 ± 0.08aA	3.68 ± 0.10a	7.39 ± 0.50bC	9.54 ± 0.31abD
300		1.91 ± 0.08aA	4.04 ± 0.14abB	7.55 ± 0.24bcC	11.37 ± 0.21bcdD
600		1.60 ± 0.21aA	2.75 ± 0.26aA	6.09 ± 0.38abB	10.01 ± 0.69abcdC
1000		2.20 ± 0.05aA	3.63 ± 0.46abA	5.71 ± 0.50aB	9.76 ± 0.42abcC
0	CH	2.50 ± 0.14aA	4.50 ± 0.50abB	6.45 ± 0.25abC	9.30 ± 0.14aD
300		2.47 ± 0.14aA	4.93 ± 0.31bcB	9.06 ± 0.82cdC	11.72 ± 0.41dD
600		2.33 ± 0.05aA	3.69 ± 0.35abB	7.62 ± 0.23bcC	10.42 ± 0.27abcdD
0	CMCH	2.37 ± 0.14aA	4.45 ± 0.37abB	10.00 ± 0.24dC	14.01 ± 0.85eD
300		2.13 ± 0.49aA	6.56 ± 0.41cA	10.68 ± 0.32dB	14.92 ± 0.08eC
600		1.65 ± 0.33aA	3.54 ± 0.62abB	7.06 ± 0.40abC	11.63 ± 0.48 cdD

The results showed that SC treatments remarkably reduced the growth of *E. coli* O157:H7 population and higher SC concentration resulted in stronger inactivation. Among all treatments, 1000 mg/L SC achieved the greatest inactivation with more than a 4.5 log CFU/g reduction over the control. SC, which has been widely used for textile bleaching, water purification, and dental hygiene, has been demonstrated to be an effective sanitizer for inhibiting microbial growth due to its powerful oxidizing property. Recent studies have also reported the antimicrobial effect of SC on fresh-cut cilantro (Allende et al., 2009) and fresh-cut 'Granny Smith' apples (Guan and Fan, 2010)

In the case of the samples treated with CH or CMCH coating alone, no significant difference in *E. coli* population reduction was observed compared with the control treatment, revealing that CH and CMCH did not show any potential to inactivate *E. coli* O157:H7. In fact, the antimicrobial activity of CH is most effective against yeasts and molds, followed by Gram-positive bacteria, and finally, Gram-negative bacteria (Rabea et al., 2003). In our work, *E. coli*, a Gram-negative bacterium, was studied, and no obvious antimicrobial effect was observed. Although there are several studies showing the effect of CH on *E. coli* inhibition (Darmadji and Izumimoto, 1994; Wang, 1992), different experimental conditions can result in different outcomes. For example, pH, temperature, and surrounding matrix are among factors that can impact the antimicrobial properties of CH. In our experiment, TSB, the medium in which the *E. coli* population was cultured, is alkaline with a pH of 7.3, a condition in which CH has poor solubility. Therefore, CH, which no longer possessed the positive surface charges that it possesses in acidic solution, was not able to interact with negatively charged residues present in the cell wall of bacteria, nor to alter the cell wall permeability and thus had no inhibitory effect on the microorganisms. Moreover, it is worth noting that no long-term microbial experiments on fresh-cut pears were carried out in our study. Therefore, the inhibitory effect of CH on microorganisms on fresh-cut pears during the storage time was not obtained.

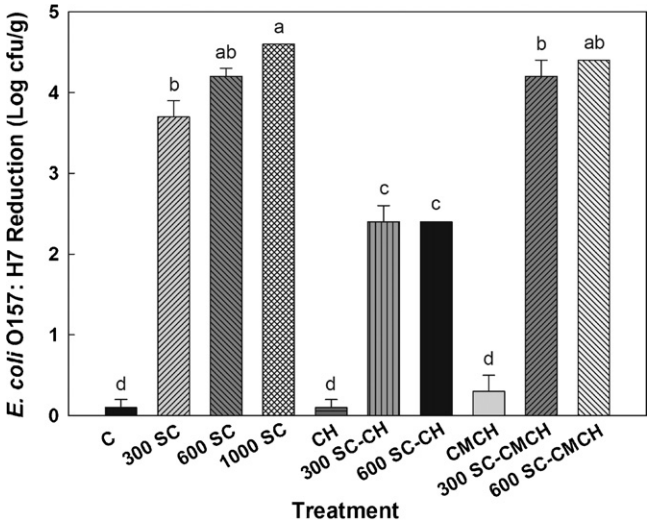

Fig. 5. Inactivation of *E. coli* O157:H7 by sodium chlorite (SC) with or without edible coatings (CH/CMCH). C is the control. Values followed by the same lower case letter are not significantly different ($P > 0.05$). Vertical bars represent standard errors ($n = 3$).

There are few reports on the antimicrobial properties of CMCH. CMCH with a molecular weight less than 5000 Da was able to inactivate *Staphylococcus aureus* (Chen et al., 2000). Recently, Zhong et al. also found that CMCH showed some antifungal activity; however, it was inactive against four bacteria tested, including *E. coli* (Zhong et al., 2009). This finding was consistent with our results.

The combined treatment of SC and CH reduced microbial populations by 2.4 log CFU/g, considerably more effectively than the control. Since the pears were treated with SC prior to CH coating, SC could directly contact the *E. coli* inoculum on the surface of pear slices, resulting in inactivation of some of the microorganisms. The subsequent CH coating treatment counteracted the effect of SC to some extent as a result of oxidation (Murinov et al., 2010). FT-IR analysis confirmed the oxidation of CH and the lack of oxidation of CMCH in the presence of SC. Therefore, the inhibitory effects of SC + CH treatment on *E. coli* growth were significantly decreased, compared to that of SC treatments. Since SC does not oxidize CMCH, the antibacterial activities of SC + CMCH treatments were similar to those of SC treatments, demonstrating a strong inhibition against *E. coli*.

4. Conclusions

Our study showed that SC possessed dual effectiveness in controlling enzymatic browning and preventing the growth of *E. coli* O157:H7 on minimally processed pears. The combination of CH with SC interfered with SC's efficacy to inhibit PPO activity and exacerbated the browning reaction on pear slices. However, the combination of SC and CMCH coating demonstrated beneficial effects in reducing cut-surface discoloration and inactivating *E. coli* O157:H7 populations. Additionally, SC and coatings prevented tissue softening and exhibited no significant effect on weight loss. Our results suggested that the treatment of SC combined with CMCH coating could be an alternative technology used for maintaining the quality and extending the shelf life of fresh-cut d'Anjou pears.

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